Research Journal of Medical Sciences 2 (1): 47-50, 2008
ISSN: 1815-9346

The Cutting Edge in Stem Cell Medical Applications

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Abstract: The steady increase in the average life-expectancy in the developed world can be attributed in part at least, to advances in virtually all areas of medicine, starting with technological advances in diagnostic techniques, greater awareness as regards living a healthy lifestyle, more efficient surgery protocols, while less invasive methods are used whenever possible, a greater depth of the etiology and pathogenesis of the disease and so on and so forth. However, despite overcoming a great number of illnesses, other formidable diseases still remain unscathed by even the most aggressive treatment. This seems to be the point in fact, where the promise of stem cells becomes more exciting and poignant. Research investigating the potential use of stem cells against these illnesses yielded a number of encouraging results and even successful therapy protocols in some instances (ex. leukaemia). Some therapies are also in the pipeline while others are still experimental. Having said this, the material available today gives an encouraging boost for all involved in the fight against some of the debilitating and ruthless illnesses of our times.

Key words: Stem cells, brain tumours, Parkinson's disease, Alzheimer's disease, organ regeneration, kidney

INTRODUCTION

Stem cells are defined as "clonogenic self-renewing progenitor cells that have the ability to divide from an indefinite period and can give rise to one or more differentiated cell types" (Vejjajiva, 2002), which ability is known as developmental plasticity.

The plasticity varies in the different types of stem cells. The zygote gives rise to all cell types and normally develops into an embryo. It is said to be totipotent. Embryonic and umbilical stem cells also give rise to all cell types. However given that they are not able to develop into an embryo, they are described as pluripotent. Finally adult stem cells show a range of plasticity from pluri-to multipotent with mesenchymal cells being able to give rise to lipocytes, cartilage, bone, tendon and ligaments, myocytes, skin cells and neurons, while haematopoeitic stem cells are able to give rise to red, white blood cells, immune cells among others. Transdifferentiation (the crossing of germ layers) is also possible.

This amazing range of plasticity of stem cells has given hope in the fight against conditions as diverse as brain tumours, neurodegenerative diseases, spinal injuries and amazingly, also in the regeneration of tissue, such as in liver and kidney, to name just two examples.

Cancer- brain tumours: As in all cancers, brain tumour cells arise from normally functioning cells which undergo mutations, leading to a change in function as well as uncontrolled proliferation of the tumour cells. Current tests for brain tumours focus on all cells, instead of specifically attacking the cells which lead to the uncontrolled growth, in other words the tumour stem cells. This could explain the poor rate of response of the tumours to treatment.

The first isolation of cancer stem cells from the central nervous system was performed by Dr. Peter Dirks and Dr. Sheila Singh, at the University of Toronto (Singh *et al*., 2003).

The approach used primary human brain tumours, obtained from neurosurgical operations, from which the team isolated and characterized a cancer stem cell from human brain tumours of different phenotypes, more specifically low grade and high grade primary brain tumours consisting of astrocytomas, glioblastomas, ependymomas, medulloblastomas, angliogliomas (Fig. 1). The isolated cells express neural stem cell markers, have stem cell-like behaviour *in vitro* and represent only a small fraction of the total tumour cell population.

Given that these cancer stem cells are necessary for maintaining tumour growth *in vitro*, their identification has important implications for understanding the mechanisms of brain tumorigenesis. Furthermore, since this cell represents only a small proportion of the total number of cells in a brain tumour (Reynolds and Weiss, 1996; Morshead *et al*., 1994) results obtained

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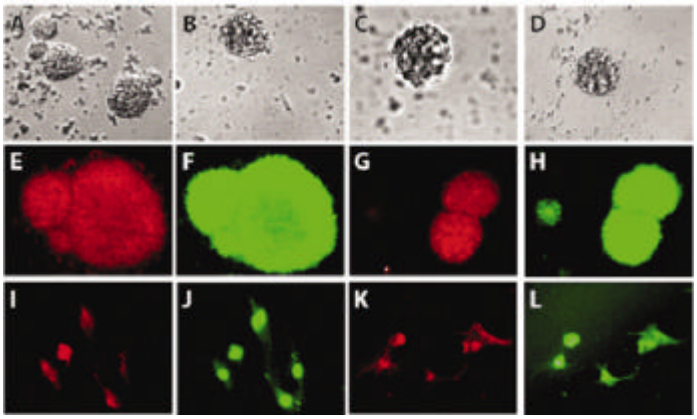

Fig. 1: Primary brain tumours of different phenotypes form neurosphere-like colonies. Photomicrographs of cultured brain tumour cells (magnification × 20) at 24-48 h after plating in TSM (Tumour Sphere Medium), containing EGF (Epidermal Growth Factor) and bFGF (basic Fibroblast Growth Factor). Each tumour subtype yielded growth of cells in neurosphere-like clusters, termed tumour spheres

suggested that therapy that spares this cell may explain tumour recurrence.

Hence, it follows, that if the said finding were to be borne out by future research, it could offer a potential new approach to fighting brain tumours, by targeting brain tumour stem cells and thus stopping the growth and spread of the cancer (Singh *et al*., 2003).

Tumour spheres are shown from a medulloblastoma (A), pilocytic astrocytoma (B), ependymoma (C) and ganglioglioma (D). Undifferentiated primary tumor spheres from a medulloblastoma (E, F, I and J) and a pilocytic astrocytoma (G, H, K and L) are immunostained at 4 h for characteristic neural stem cell marker nestin (E and G) and for CD133 (F and H). Individual undifferentiated medulloblastoma sphere cells and astrocytoma sphere cells are also shown stained for nestin (I and K) and CD133 (J and L) (Singh *et al*., 2003).

NEURODEGENERATIVE DISORDERS

Parkinson's Disease (PD): PD seems to be ideal for stem cell treatment, given that the main pathology is a relatively selective degeneration of a specific type of neuron, the nigrostriatal dopamine neuron. This is as opposed to ischeamic disease, as in for example stroke, or Alzheimer's disease.

Neural Stem Cells (NSCs) exhibit high potencies of self-renewal and neuronal differentiation. Hence, various research projects have been conducted in order to try to understand in greater depth the complex processes involved in neuronal development and ultimately to find a cure for PD. Thus, in the project mentioned above, Yasuhara and her team tried to establish whether transplantation of human NSCs cloned by v-myc gene transfer, HB1.F3 cells, is a feasible therapeutic option for Parkinson's disease. This was done by transplanting green fluorescent protein-labelled HB1.F3 cells (200,000 viable cells in 3 microl of PBS) *in vivo*, into the 6-hydroxydopamine-lesioned striatum of rats. It was in fact established that this procedure significantly ameliorated parkinsonian behavioural symptoms, when compared with controls (vehicle, single bolus, or continuous minipump infusion of trophic factor, or killed cell grafts).

In another project, mesencephalic precursor cells were studied in more detail, given that to date, the generation of dopaminergic neurons from mesencephalic precursors has been difficult to follow, partly because an appropriate means for recognizing mesencephalic ventricular zone precursors has not been available. To overcome his difficulty, Sawamoto's team used transgenic mice and rats carrying Green Fluorescent Protein (GFP) cDNA under the control of the nestin enhancer, in order to visualize and isolate mesencephalic precursor cells from a mixed population. From the results, it can be seen that nestin-driven GFP was detected in the mesencephalic ventricular zone. Additionally, data obtained from flow-cytometry indicated that Prominin/CD133, a cell-surface marker for ventricular zone cells, was expressed specifically in these GFP-positive (GFP(+)) cells. These cells were cultured *in vitro*, after sorting by fluorescence-activated cell sorting. The sorted population was shown to be able differentiate into both neurons and glia. Furthermore, many neurons generated from

nestin-GFP-sorted mesencephalic precursors developed a dopaminergic phenotype *in vitro* and were, in fact, were able to restore dopaminergic function in the host striatum, as assessed by a behavioral measure: recovery from amphetamine-induced rotation, when they were transplanted into the striatum of a rat model of Parkinson's disease (Sawamoto *et al*., 2001).

Both these functions indicate that stem cells give promising results in providing treatment for PD when used in protocols in the lab.

Alzheimer's Disease (AD): To date, much evidence has become available, to the effect that the brain is capable of regenerating neurons after maturation. In a recent study, Human Neural Stem Cells (HNSCs) transplanted into aged rat brains differentiated into neural cells. Furthermore, they significantly improved the cognitive functions of the animals, indicating that HNSCs may be a promising candidate for cell-replacement therapies for neurodegenerative diseases including AD (Sugaya *et al*., 2006). However, HNSC have been a source of ethical controversy, leading the same group of researchers to explore novel technologies to differentiate adult human mesenchymal stem cells, a subset of stromal cells in the bone marrow, into neural cells by modifying DNA methylation or over expression of nanog, a homeobox gene expressed in embryonic stem cells. The study, which was published some time after, reported that peripheral administrations of a pyrimidine derivative that increases endogenous stem cell proliferation, improves cognitive function of the aged animal (Sugaya *et al*., 2006).

Although these results may promise a bright future for clinical applications used towards stem cell strategies in AD therapy, it is important to keep in mind the fact that AD is complex, as all pathological processes. Hence, while these results are encouraging, more work needs to be done before it can be successfully treated.

Spinal injury: Impaction of the spinal cord results in immediate damage to various tissue types, including blood vessels, as well as nercous tissue, causing loss of neurons, astrocytes and oligodendrocytes. In time, secondary injury may result causing more tissue nearby or away from the injury site to be lost. Although, in case of relatively minor damage to the cord, some return of function can be observed, in most cases the neurological loss is permanent.

Stem cells have a high potential for regeneration and have in fact, been implicated in repair of the adult spinal cord. *in vitro* and *in vivo* studies on the use of BMSCs for repair of the spinal cord have been conducted in experimental injury models and their potential for human application was studied (Nandoe *et al*., 2006).

In another research project, given that the injured adult spinal cord is not conducive to neuronal regeneration and neurogenesis, attempts were made to enhanced neurogenesis or oligodendroglia differentiation of transplanted Neural Precursor Cells (NPCs) by genetic manipulation, such as for example the exogenous expression of noggin, with the idea of antagonizing the astroglia differentiation promoting Bone Morphogenetic Proteins (BMPs). Direct attempts to enhance neurogenesis have also been made in transgenic over-expression of neurogenic basic helix-loop-helix transcription factors (Tang and Law, 2007). Having said this, it is important to keep in mind that successful repair of the spinal cord requires the development of *in vitro* techniques that will generate the desired cell type and/or a large enough number for *in vivo* transplantation approaches.

ORGAN REGENERATION

Kidney: Unlike in other areas (as in leukaemia for example), in nephrology the use of stem cell therapy has yet to reach practical therapeutics applications. In fact, no one as yet has coaxed bone marrow stem cells to become renal cells although the local presence of renal adult stem cell-like cells has been confirmed (Humes *et al*., 1999). It sees that while the initial work by Humes and his colleagues in Ann Arbor Michigan USA in the years 1996-2001 enjoyed some degree of success, in the last few years the interest and promise have waned. Having said this, it is worth noting that Humes and colleagues were able to build a biosynthetic renal tubule assist device where the synthetic fibres were coated with porcine tubular cells that appeared to mimic certain biochemical properties of the human kidney, such as the synthesis and activation of vitamin D (Humes *et al*., 1999).

Poulsom (2001) examined kidneys of female mice that had received a male bone marrow transplant and kidney biopsies from male patients who had received kidney transplants from female donors, in order to establish whether extra-renal cells contribute to the turnover and repair of renal tissues. The method used *in situ* hybridization to detect Y-chromosomes, after which it could be demonstrated that circulating stem cells frequently engraft into the kidney and differentiate into renal parenchymal cells.

When this was applied to human renal grafts, it was confirmed that some of the recipient-derived cells within the kidney exhibited a tubular epithelial phenotype, by combining *in situ* hybridization with immunostaining for the epithelial markers CAM 5.2 and the lectin *Ulex europaeus*. Furthermore, female mouse recipients of male bone marrow grafts showed co-localization of Y-chromosomes and tubular epithelial markers *Ricinus communis* and *Lens culinaris* and a specific cytochrome P450 enzyme (CYP1A2) indicating an appropriate functional capability of clustered newly formed marrow-derived tubular epithelial cells. Y-chromosome-containing cells were observed within glomeruli, with morphology and location appropriate for podocytes, while within the murine kidney, these Y-chromosome-positive cells were negative for the mouse macrophage marker F4/80 antigen and leukocyte common antigen, but were vimentin-positive. Bone marrow-derived cells were present in both histologically normal mouse kidneys and in human transplanted kidneys suffering damage from a variety of causes. This data indicates that bone marrow cells contribute to both normal turnover of renal epithelia and regeneration after damage (Poulsom *et al*., 2001).

More recently, research performed at Yale and the London hospitals confirming the importance of the bone marrow in tubulogenesis and the recovery of acute renal failure have provided indirect evidence to the presence of potential renal cells in the bone marrow and may open the door to harvesting such cells for future use, given the fact that data from experimental animal models of renal diseases have shown that infused bone marrow-derived SC may repair the injured glomeruli (Schena and Abbattista, 2003).

At present the production of a biosynthetic human kidney to treat patients with chronic renal failure remains a dream.

CONCLUSION

While on the one hand the promise of stem cells is undeniable, especially given the fantastic results achieved experimentally, it is important to note that a lot needs to be done before most of the therapies based on stem cells are available on the market.

Illnesses like brain tumours, renal failures and neurodegenerative disorders seem set to continue to feature prominently in the years to come as the population continues to age. Hence, it is vital for solutions to be researched thoroughly in the interest of patients, their relatives and ultimately the whole of humanity.

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